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# Factors Responsible for the Distribution of Mycorrhizal Fungi of *Pinus* in Eastern Australia

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*Abstract*

Mycorrhizal fungi were isolated directly from pine roots. Individual plantations yielded up to 42 species from *Pinus elliottii* Little & Dorman, and 20 from *P. radiata* D. Don, depending on the locality. The mycorrhizal flora varied seasonally, with a decline in numbers of species during the winter. Seedlings that survived after direct sowing trials of *P. elliottii* yielded from one to 11 species, depending on locality, proximity to plantations and subsequent inoculation. Nursery stock yielded only four species from *P. radiata* and the same number from *P. elliottii*. Isolations from natural regeneration showed a rapid decline in the number of mycorrhizal species as the distance from plantations increased. Fungi from plants greater than 800 m from plantations were identified as either *Rhizopogon roseolus* (Corda), T. M. Fr. or *Suillus luteus* L. ex Fr., S. F. Gray. It is inferred that the dominance of basidiospore-producing fungi on plants at a distance from the plantation is a result of the greater survival potential and more efficient dispersal mechanism of basidiospores. Isolations from 1–5-year-old plantation stock showed that a succession of mycorrhizal species replaced those present when seedlings left the nursery.

[O.D.C. 181.351 : 174.7 *Pinus* spp. : (941)]

## Introduction

The beneficial effects of ectomycorrhizas on pines, and their significance for successful plantation establishment have been demonstrated in many parts of the world (Mikola 1973), and this has led to a great deal of research on pure culture inoculation. This research has mainly utilized fungi that form conspicuous fruiting bodies in plantations and that have been regarded as the most important species that form mycorrhizas. Zak and Bryan (1963), Zak and Marx (1964) and Lamb and Richards (1970) concluded that many more mycorrhizal species were present than commonly fruit, and that the species that were usually sighted may be only infrequently associated with mycorrhizas in plantations. Little is known of the ecology of mycorrhizal fungi either in the root or in the soil (Bowen and Theodorou 1973), or of the influence of climate, site or time on the mycorrhizal flora. Such information is necessary to the establishment of an inoculation program as envisaged by Bowen (1965) and Mikola (1970).

In the 1960s the New South Wales Forestry Commission conducted a number of direct seeding trials with *P. elliottii* Little & Dorman in coastal New South Wales. Most of these trials failed as a result of a lack of appropriate mycorrhizal fungi in the soil, even though some trial plots were adjacent to established pine plantations. This paper describes a series of investigations into the factors that influence the distribution of ectomycorrhizal fungi of *Pinus* in these and other areas of eastern New South Wales and south-eastern Queensland.

## Experimental Procedures

Mycorrhizal roots were collected from the decomposing organic matter at the base of the litter layer and the top 10 cm of mineral soil, and individual samples placed in plastic bags which were transported under refrigeration to the laboratory. After initial washing under tap water individual mycorrhizas were excised and shaken for 10 min in a solution of 0.1% w/v non-phytotoxic detergent. They were then surface sterilized for 10 min in 1% w/v calcium hypochlorite solution, rinsed with sterile water, and plated out on Hagem agar.

Fungi were tested for mycorrhiza formation as follows. Pine seed was surface sterilized in 30% v/v hydrogen peroxide, plated out for 4 days onto nutrient agar to check sterility, and then germinated in sterile peat/vermiculite. Seedlings were transplanted into 3 X 18 cm pyrex tubes plugged with glass wool and containing sterile peat/sand (1 : 4 v/v). Each tube stood in a steel tray containing mineral nutrient solution.

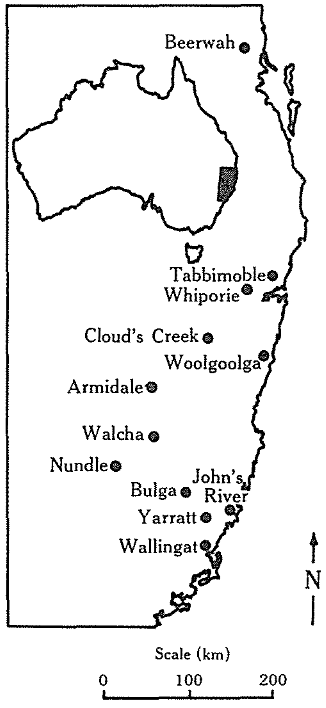

Fig. 1. Part of eastern Australia showing the location of state forests and nurseries referred to in the text. The shaded area on the inset shows the area represented.

Tubes were inoculated at planting with discs of agar containing fungal mycelia from plates, and the surface of the rooting medium was covered with sterile polyethylene chips. Tubes were watered from below with sterile water, and seedlings grew for 4 months in a controlled environment cabinet under a 16-h photoperiod at temperatures of 24.2±2°C (light) and 17.2±2°C (dark). Mycorrhizal formation was assessed by microscopic examination of thin sections, and fungi were reisolated as described above. (For further detail of methods, see Lamb and Richards 1970.)

Isolations were made from all fruiting bodies found in the study areas as an aid to the identification of isolates from roots. The locations of the state forests and nurseries that are mentioned in the text are shown in Fig. 1. Isolation series were taken from the following sources.

*Mature plantations.* Mature stands of *P. elliottii* and *P. radiata* D. Don were sampled over a 4-year period; *P. radiata* at Armidale and *P. elliottii* at Cloud's Creek, Tabbimoble and Whiporie State Forests. Every 2 months 100 samples were collected from each locality so that an even distribution with respect to stand age was obtained. The samples from each locality were bulked in the laboratory and the mycorrhizal fungi isolated.

*Seedlings surviving in direct sowing trials.* Trial plots used by the N.S.W. Forestry Commission at Tabbimoble, Whiporie, John's River, Bulga, Wallingat and Yarratt State Forests and sown with *P. elliottii* were sampled every 6 months for 5 years beginning in March 1970. Inoculated seedlings that had been planted artificially to restock blank areas were avoided where possible.

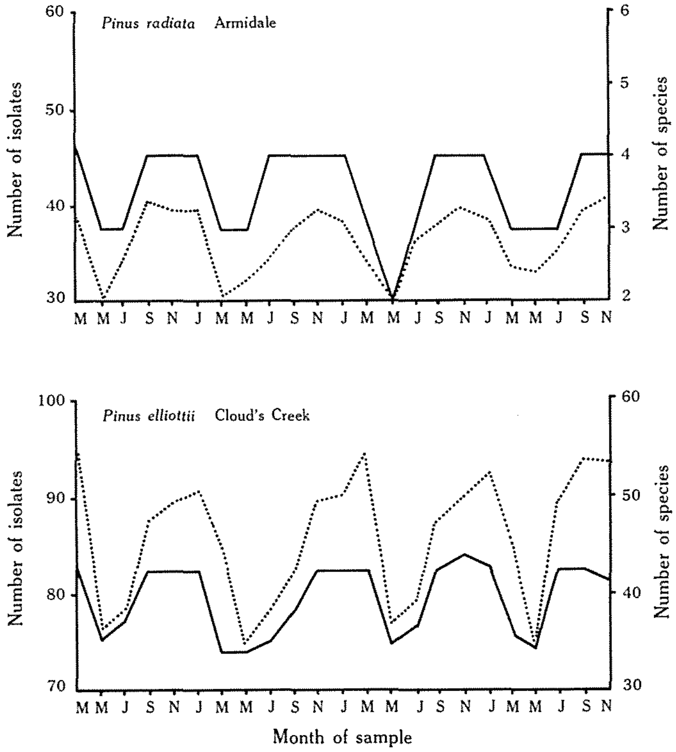

**Fig. 2.** Seasonal variations in the number of isolates and putative species of mycorrhizal fungi obtained from *P. radiata* and *P. elliottii* at two tableland localities over a 4-year period. ....., number of isolates (left axis); ——, number of putative species (right axis).

*Nursery stock.* Nursery seedlings of *P. radiata* at Walcha and Nundle on the northern tablelands of New South Wales, and *P. elliottii* at Woolgoolga (N.S.W. north coast), and Beerwah (coastal south-eastern Queensland) were sampled about 2 months before planting-out; 100 samples were taken from each locality.

*Natural regeneration near plantations.* Samples of roots from natural regeneration were taken at intervals of 6 months for 5 years beginning in March 1971 at Armidale and John's River (*P. radiata*), and Tabbimoble, Whiporie and Cloud's Creek State Forests (*P. elliottii*). At each locality 10 samples were taken from plants in each of five distance classes from the margin of the established plantation, viz. 0-200 m, 200-400 m, 400-800 m, 800-1000 m and 1-2 km. Samples from each distance class at each locality were bulked in the laboratory before processing.

*Plantation stock 1-5 years old derived from previously sampled nurseries. P. radiata* planted out from Walcha nursery and *P. elliottii* from Woolgoolga were sampled every year for 5 years with 200 samples taken each year. *P. elliottii* was sampled at Cloud's Creek and Whiporie, and *P. radiata* at Nundle.

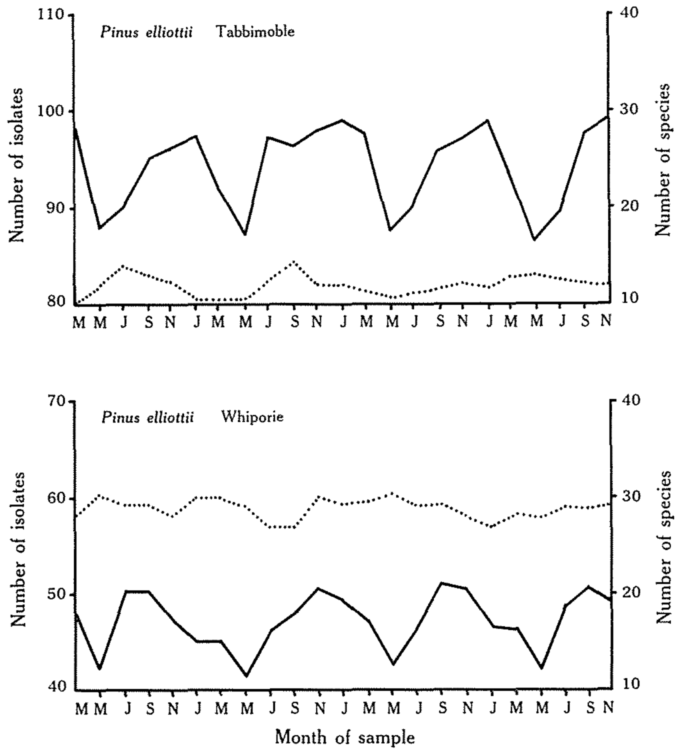

**Fig. 3.** Seasonal variations in the number of isolates and putative species of mycorrhizal fungi obtained from *P. elliottii* at two coastal localities over a 2-year period. ....., number of isolates (left axis); ——, number of species (right axis).

## Results

### *Seasonal Variations in Isolates from Mature Plantations*

Figs 2 and 3 show the numbers of basidiomycete isolates and putative species obtained from sampling every 2 months for 4 years at four sampling sites. During the 4-year sampling period only four fungal species were derived from *P. radiata* at Armidale, but *P. elliottii* yielded a total of 44 species; 42 at Cloud's Creek, 30 at Tabbimoble and 20 at Whiporie. Two mycorrhizal species for *P. elliottii* were isolated only at Whiporie. Of the total of 48 species, 46 formed mycorrhizas on the host from which they were derived; only two isolates from *P. elliottii* failed to form mycorrhizas under the test conditions.

At Armidale and Cloud's Creek the numbers of isolates were lowest in May and July; the two samples taken during the winter months. This result was not shown by the samples from the two coastal localities Tabbimoble and Whiporie. The number of species isolated, however, showed a marked reduction from late summer (March) to early winter (May) at all localities, and remained low in July at Armidale and Cloud's Creek.

### *Isolations from Seedlings Surviving in Direct-sowing Trials*

Table 1 shows the total number of basidiomycete isolates and putative species obtained from seedlings surviving in trial areas of direct sowing in six localities.

**Table 1. Total number of basidiomycetes and number of putative species isolated from mycorrhizal roots of *P. elliottii* seedlings in areas used in direct sowing trials**

| Locality | | No. of isolates | | No. of species |
|---|---|---|---|---|
| Tabbimoble | | 194 | | 11 |
| Whiporie | | 176 | | 2 |
| John's River | | 200 | | 4 |
| Bulga | | 182 | | 3 |
| Wallingat | | 230 | | 2 |
| Yarratt | | 245 | | 1 |
| | Total isolates | 1227 | Total species | 12 |

A much smaller number of mycorrhizal species was isolated from direct-sown areas than from routine plantations (cf. previous section on Seasonal Variation). Samples from Tabbimoble yielded a total of 11 species, which was the largest number for any direct sown area. However, most of these species were isolated from plants that had been inoculated with soil from a healthy plantation after sowing trials had initially resulted in non-mycorrhizal seedlings. At the other five localities from one to four species only could be isolated and of these three were isolated only rarely. None of the species isolated was identifiable using cultures derived from sporophores of suspected formers of mycorrhiza. One species was isolated from all localities. Mycorrhizas from which this fungus was isolated were brown in colour with no macroscopically visible mantle, and were frequently irregularly branched. Microscopic examination of sections showed extensive intercellular penetration of hyphae typical of the ectendotrophic type as described by Wilcox (1971).

### *Isolations from Nursery Stock*

The results of isolations from mycorrhizas of nursery stock are shown in Table 2. In contrast to results from plantation isolations, all the species forming mycorrhizas on nursery stock could be identified by hyphal fusion tests using cultures of known taxonomic status.

Seven species of fungi were isolated with all localities providing the same fungal species from each host species; only *R. roseolus* was isolated from both hosts. *S. granulatus* and *S. luteus* were isolated regularly but in very small numbers from nursery seedlings, whereas the gasteromycetes *R. luteolus, R. roseolus* and *P. tinctorius*, and the two species of *Thelephora*, were isolated in large numbers. All mycorrhizas that formed in the nursery were of typical ectotrophic type, unlike the ectendotrophic structures described as common in nurseries by Mikola (1965) and Wilcox (1971).

Table 2. Number of basidiomycete isolates and species obtained from nursery stock at four localities

| Locality | Host species | No. of species | No. of isolates | Fungal species |
|---|---|---|---|---|
| Walcha | *P. radiata* | 4 | 230 | *Rhizopogon luteolus* Fr. & Nordh |
| Nundle | | 4 | 284 | *R. roseolus* (Corda) T. M. Fr. |
| | | | 38 | *Suillus luteus* L. ex Fr. S. F. Gray |
| | | | 387 | *Pisolithus tinctorius* Pers. Coker & Couch |
| Woolgoolga | *P. elliottii* | 4 | 158 | *Thelephora laciniata* Pers. |
| | | 4 | 246 | *R. roseolus* (Corda) T. M. Fr. |
| | | | 43 | *S. granulatus* L. ex Fr. |
| | | | 207 | *T. terrestris* (Ehrh.) Fr. |
| | Total species | 7 | Total isolates 1593 | |

*Isolations from Natural Regeneration near Plantations*

The results of this investigation are shown in Fig. 4. A rapid decline in the number of fungal species is seen, beginning as little as 200 m from the established plantation for *P. elliottii*. At Whiporie no mycorrhizal fungi for *P. elliottii* could be isolated at a distance greater than 600 m. Volunteer plants at Cloud's Creek, on the other hand, contained mycorrhizal fungi at distances in excess of 1 km, but at 2 km none could be isolated.

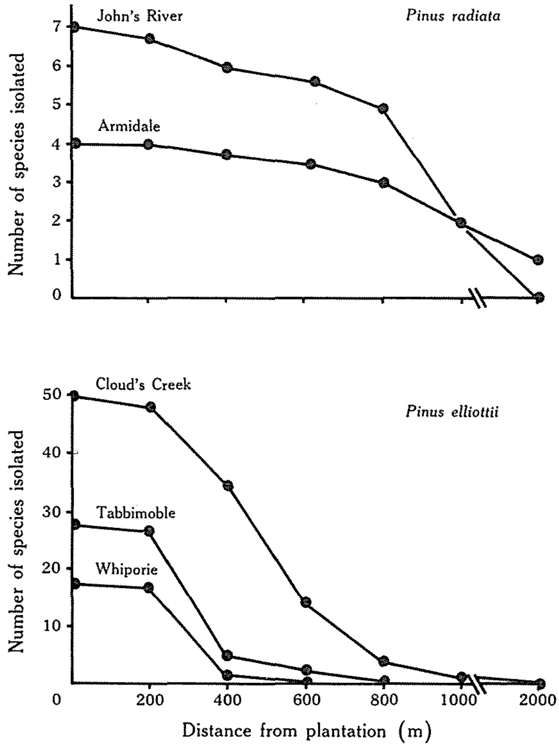

Fig. 4. Effect of distance from the nearest plantation edge on the number of putative mycorrhizal species isolated from roots of natural regeneration of *P. radiata* and *P. elliottii* at five localities.

For *P. radiata* the decline in the number of species was less rapid, but the number present was much lower. At Armidale, where *P. radiata* is naturalized, volunteer plants in excess of 2 km from the plantation were mycorrhizal and gave rise to one fungal species, *R. roseolus.* At John's River, however, in an area where *P. radiata* has not become naturalized, numbers of fungi declined to zero between 1 and 2 km from the plantation. The two fungal species found at distances exceeding 800 m were identified as *R. roseolus* and *S. luteus.* The single fungal species that could be isolated from volunteer *P. elliottii* at distances greater than 800 m from the plantation at Cloud's Creek was *R. roseolus.*

### *Isolations from 1-5-year-old Plantation Stock Derived from Previously Sampled Nurseries*

The results are shown in Table 3. A year after planting out both pine species retained their nursery flora of mycorrhizal fungi, but by the end of 5 years this had been replaced by a larger number of unidentified species similar to that in the surrounding mature plantation. The Nundle plantation of *P. radiata* yielded 20 mycorrhizal species, compared with seven at Armidale (cf. the section on Seasonal Variation).

**Table 3. Number of basidiomycete isolates and putative species obtained from 1–5-year-old plantation stock planted out from Walcha (*P. radiata*) and Woolgoolga (*P. elliottii*) nurseries**

| Host species | Host age (years after planting) | No. of isolates | Number of fungal species | | |
|---|---|---|---|---|---|
| | | | Common to field and nursery floras | Not previously isolated (cumulative) | Total |
| *P. radiata* | 1 | 104 | 4 | – | 4 |
| | 2 | 127 | 4 | 2 | 6 |
| | 3 | 154 | 2 | 6 | 8 |
| | 4 | 216 | 1 | 18 | 19 |
| | 5 | 234 | 0 | 20 | 20 |
| *P. elliottii* | 1 | 140 | 4 | – | 4 |
| | 2 | 283 | 3 | 8 | 11 |
| | 3 | 230 | 2 | 22 | 24 |
| | 4 | 321 | 2 | 33 | 35 |
| | 5 | 308 | 0 | 42 | 42 |

### *Synthesis of Mycorrhizas Using Fungal Isolates*

During the investigations described here 80 putative species were tested for mycorrhizal formation on both host species. The results are summarized in Table 4.

**Table 4. Results of mycorrhiza-synthesis experiments using mycorrhizal fungi isolated from pine roots**

| Source of isolates | No. of fungi tested | No. of fungi forming mycorrhizas on | | No. of successful reisolations | No. confirmed by hyphal fusion |
|---|---|---|---|---|---|
| | | *P. radiata* | *P. elliottii* | | |
| *P. radiata* | 20 | 20 | 18 | 20 | 13* |
| *P. elliottii* | 60 | 42 | 57 | 57 | 50* |
| Total no. of fungi tested | 80 | Total no. of mycorrhiza forming species | 77 | | |

* The remainder were confirmed on the basis of morphological characteristics.

Of the total number of species tested during the investigation only seven could be identified using cultures of known taxonomic status. A further 70 species were shown to be capable of forming mycorrhizas on either one or both host species. All but three fungi isolated from *P. elliottii* were capable of forming mycorrhizas on that species, but two from *P. radiata* did not form them on *P. elliottii* and 15 from *P. elliottii* did not produce mycorrhizas on *P. radiata.*

## Discussion

These investigations confirm that a very large number of mycorrhizal fungi are present in the soil, and those that form conspicuous fruiting bodies and have been regarded as major mycorrhiza formers may play a relatively minor role in mycorrhizal symbioses in plantations. Since only basidiomycetes bearing clamp connections were recorded, and since only fungi capable of growth under the test conditions were isolated, only a proportion of the total number of species may be represented here. The results further indicate that the mycorrhizal flora varies not only from one locality to another on each pine species, but also seasonally. This raises the questions of where and in what form those fungi that do not overwinter in the mycorrhizal association survive. In some tableland localities isolation rates are lower in winter when root growth ceases, thus accentuating the reduction in number of species isolated, but in coastal localities where milder winters permit some root growth to occur the decline in species numbers is still evident. It is postulated that either these fungi possess sufficient saprophytic ability to survive the winter in the soil, or else produce resting structures that perform this function. Some of these species have been shown both to be less fastidious in their carbon nutrition than better known fungi (Lamb 1974) and to produce chlamydospores with a limited survival potential (Lamb and Richards 1974). Warcup (1959) isolated fungi similar to those forming mycorrhizas on *P. radiata* from dead or decaying grass roots nearby, and at least one mycorrhizal fungus of *P. radiata* can colonize the rhizosphere of grasses (Theodorou and Bowen 1971). If growth in the rhizosphere of non-host plants is important then this could provide a reservoir of mycorrhizal fungi over the winter and in the absence of host plants. Bowen (1965) showed that mycorrhizal fungi for *P. radiata* occurred naturally in some 50% of soils sampled in South Australia not in close proximity to pine plantations, and speculated that they might exist in association, not necessarily mycorrhizal, with *Eucalyptus* spp. Presumably those fungi that remain in the mycorrhizal association during winter would be more important in the initial infection of new short roots formed when root growth recommences in spring. However, the relative importance of both groups of fungi to the overall well being of the host plant is difficult to ascertain. Marks and Foster (1967) showed evidence of replacement of mycorrhizal fungi in individual roots of *P. radiata* by different species, and concluded that an interruption of root growth was necessary before replacement occurred. If such interruptions occur in winter in plantations in eastern Australia these observations may help to explain seasonal variations in the mycorrhizal flora.

The rapid decline in numbers of mycorrhiza-forming fungi with increasing distance from plantations probably accounts for the failure of direct-sowing trials of *P. elliottii* on the north coast of New South Wales. The restricted range over which fungi are distributed is surprising since it has been assumed that the drift of basidiospores (Robertson 1954) from fruiting bodies of mycorrhizal fungi in established stands is an important source of mycorrhizal species (Bowen 1965). Such spores could be expected to retain viability after dispersal over quite large areas. Many of the fungi isolated are assumed to fruit rarely, if at all, and are therefore dispersed by means other than basidiospores, e.g. as somatic hyphae or in a vegetative resting stage. This might account for the restricted dispersal since such propagules have been shown to have little survival value (Lamb and Richards 1974). It is likely also that seedlings become less susceptible to infection once chlorosis sets in and growth ceases (Hatch 1937; Richards 1965). Only fungi capable of spreading as basidiospores were found infecting seedlings at distances greater than 800 m from plantations; these fungi have an advantage in such situations because of the more efficient dispersal mechanism and survival potential of their spores.

In the nursery, the better known mycorrhizal fungi are dominant, but after transfer into the field they are replaced by a diverse unidentified mycoflora. It is inferred that these unknown fungi are not present in native forest soils and must therefore have been introduced with the seedlings at planting, i.e. in the soil surrounding seedling roots but not in the mycorrhizal association (nurseries from which isolations were made were originally inoculated with soil and litter from mature plantations). This lends further weight to the belief that such fungi possess saprophytic ability outside the host root, even if restricted to the rhizosphere of pine seedlings.

The replacement in time of an entire mycorrhizal flora demonstrated here for the first time has great implications for artificial inoculation programs, where the persistence of a particular pure culture inoculum must be assured if the benefits of inoculation are to be maintained. Further work on competition between and succession of mycorrhizal species of *Pinus* is necessary if the selection of effective inoculants is to become a reality.

## Acknowledgments

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